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STUDIES TO EVALUATE REDUCED OXIDATIVE STRESS AS A MECHANISM OF ACTION OF ANTICATARACT EFFECTS OF LIPOSOMAL FORM OF MAGNESIUM TAURATE

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Dissertation submitted in partial fulfillment of the requirements
For the degree of
MBBS with Advance Medical Science

October 2013

Acknowledgements

Firstly, I would like to say Alhamdulillah and outmost grateful to Allah S.W.T for giving me chance to complete this project and present this thesis.

I am sincerely thankful to my supervisor, Associate Professor Dr. Renu Agarwal, who is an amazing mentor and my main advisor. I thank her for always being there to support me throughout my research project with her patience and giving all her knowledge to me. She inspired and motivated me to do this project. Also, I would like to appreciate for all the help, suggestions and ideas provided by my co-supervisors, Assoc. Prof. Dr. Igor Iehitsza and Prof. Dr. Nafeeza Mohd Ismail. I am taking this opportunity to thank Dr. Nurul Alimah Abdul Nasir and Dr. Norhafiza Ramli for all the guidance they provided during this research.

Apart from that, I wish to express my sincere gratitude to the Dean of Faculty of Medicine of UiTM, Prof. Dato' Dr. Khalid Haji Yusoff, and also to coordinator of MBBS with Advance Medical Science programme, Prof. Dr Mohammed Nasimul Islam for offering this programme to all of us. It gave us an opportunity to participate and learn everything about research that was really a new thing for us. Acknowledgment must also go towards UiTM for funding this project through Dana Kecemerlangan 600-RMI/DANA-5/3/RIF (491/2012) and also Fundamental Research Grant Scheme (FRGS) grant 600-RMI/ST/FRGS 5/3/Fst (50/2011).

I also would like to take this opportunity to thank both of my parents, and Mrs. and also to all my siblings, my brother in law and sister in law for encouraging me to take this opportunity for doing research. I would like to express my gratitude to Prof. Dr. Gabriele Ruth Anisah Froemming, Director of Institute of Medical Molecular and Biotechnology (IMMB), Faculty of Medicine, UiTM Sungai Buloh, and Prof. Zainal, Director of Laboratory Animal Care Unit

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Abstract

Cataract, the lenticular opacities, develop due to increased lens oxidative stress and altered ionic balance. Our previous studies showed that magnesium taurate in aqueous form delay the development of galactose-induced cataract. However, these effects were more pronounced in vitro indicating significant permeability barriers for absorption of magnesium taurate. In the current study, we evaluated the anticataract effects of magnesium taurate loaded liposomes in comparison with its aqueous preparation and studied its effects on lenticular oxidative stress. In study 1, among the 5 groups of rats (n=9), group 1 received normal diet, while groups 2-5 received 25% galactose diet. Groups 4 and 5 received aqueous and liposomal magnesium taurate respectively, while groups 2 and 3 received corresponding vehicles. Cataract progression was assessed by weekly slit lamp examination over 28 days. The formulation showing better efficacy was chosen for study 2 in which among the 4 groups of rats (n=18), group 1 received normal diet and groups 2-4 received 25% galactose diet. Group 2 received vehicle; groups 3 and 4 received magnesium taurate and taurine respectively for 28 days. Cataract progression was assessed weekly. Subsequently, lenticular reduced glutathione (GSH), activity of superoxide dismutase (SOD) and catalase, malondialdehyde (MDA), inducible nitric oxide synthase (iNOS) protein, nitrotyrosine and proteins were measured using ELISA. Ca^{2+}/Mg^{2+} ratio was analyzed using spectrophotometer. In study 1 the liposomal magnesium taurate showed higher anticataract efficacy compared to aqueous formulation and, therefore, was chosen for study 2. In study 2, group 3 showed significantly greater delay in the cataract progression compared to groups 2 and 4. Lenticular GSH, was significantly higher in group 3 compared to both groups 2 and 4. SOD and catalase activities were restored to normal in both treatment groups, however, catalase activity remained significantly lower in group 4 compared to group 3. MDA, nitrotyrosine and iNOS protein had significantly lower values in in group 3 compared to group 2. All the parameters indicated that treatment with either magnesium taurate or taurine restores lens redox status, however, this effect was more pronounced in magnesium taurate group. Furthermore, Lenticular Ca^{2+}/Mg^{2+} ratio as well as lenticular soluble to insoluble protein ratio in both treatment groups, particularly in group 3, were restored to normal. In conclusion, topically applied liposomal magnesium taurate delays the onset and progression of the cataract more than its aqueous preparation in galactose-fed rats and this effect is attributed to the restoration of the lens redox status.

CHAPTER 1: Introduction

Cataract is an ocular disease characterized by development of opacities in the lens. These lenticular opacities impair the refraction of light on the retina, which is the normal physiological function of the lens and thereby cause visual impairment and blindness. According to an estimate by World Health Organization, total number of people from all ages with visual impairment amounts to 285 million, of whom 39 million are blind. Among the causes of visual impairment, cataract is the leading cause. It is the cause of blindness among 51% of visually impaired world population (Pascolini & Mariotti, 2012).

Various factors such as ageing, diabetes, ultraviolet radiation, smoking, dietary factors and exposure to heavy metals can cause development of cataract. Aging is suggested to be a major risk factor but the incidence of cataract formation increases further in diabetic patients (Vinson, 2006). Studies have shown that diabetic patients under the age of 65 years have three to four fold higher prevalence of cataract, while those below 65 years have two fold increase (Ederer F, Hiller R, & HR., 1981; Javadi & Zarei-Ghanavati, 2008).

Besides other factors, magnesium deficiency can also cause cataract formation Magnesium deficiency may result from several pathological conditions such as diabetes mellitus, alcoholism, cardiovascular diseases, kidney diseases, severe diarrhea and vomiting, diuretics and others (Agarwal, Iezhitsa, Agarwal, & Spasov, 2012). Magnesium is important in maintaining the ionic balance and its deficiency can increase the level of cellular oxidative stress by increasing the nitric oxide production. Excess of nitric oxide causes depletion of adenosine triphosphate synthesis (ATP), which then alters the functions of ATPases. Furthermore, functions of several ATPases are magnesium-dependent and in the presence of magnesium deficiency their functions are altered. ATPases are membrane associated transporters that play an important role in maintaining the cellular ionic homeostasis (Okuma, Uehara, & Nomura, 1998; Yasuda, Fujimori, & Panhou, 1998).

Deficiency of magnesium also affects ATPase functions by increasing lens oxidative stress. The important defenses against oxidative stress in lens include reduced glutathione (GSH), superoxide dismutase (SOD) and catalase. Magnesium deficiency is also known to enhance expression of inducible nitric oxide synthase (iNOS), an enzyme that produces excessive quantities of nitric oxide